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Entalbostroma erumpens gen. et sp. nov. (*Xylariaceae*) from *Phormium* in New Zealand

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Abstract—A xylariaceous fungus on the dead leaves of *Phormium tenax* and *P. cookianum* is described as a new genus and species, *Entalbostroma erumpens*. Phylogenetically *E. erumpens* is close to *Xylaria amphithele*. The sexual state resembles *Kretzschmariella* in general morphology, but the two genera are phylogenetically distant. The asexual state can be used to distinguish the genera morphologically, *Entalbostroma* having an anamorph with typical xylariaceous conidia, while *Kretzschmariella* has *Mirandina*-like conidia, unknown in any other xylariaceous taxon.

Key words— phylogeny, *Xylariales*, Xylarioideae

Introduction

The generic taxonomy of the *Xylariaceae* remains uncertain. Phylogenetic studies have shown that few of the long recognised, morphologically based genera form well resolved, monophyletic clades, most being polyphyletic, paraphyletic, or with poorly resolved relationships in DNA based phylogenetic studies (Hsieh et al. 2010, Pažoutová et al. 2010, U'Ren et al. 2016) As discussed by U'Ren et al. (2016), resolution of phylogenetically robust genera within the *Xylariaceae* requires not only additional genes but also more complete taxon sampling.

In this paper we fill a gap in the taxon sampling of these fungi by describing a morphologically unusual xylariaceous fungus from leaves of the monocotyledonous *Phormium* spp. (*Asparagales*, *Hemerocallidaceae*) as a new genus and species. Morphologically the sexual state is similar to

Kretzschmariella (Ju & Rogers 1994); however newly obtained DNA sequences for *Kretzschmariella culmorum* show the two taxa to be phylogenetically distant.

Materials & methods

Specimens were collected during general surveys of fungi associated with the native plants of New Zealand.

Ascospore masses were removed aseptically from perithecia in the holotype specimen PDD 107494 and spotted onto SMEA medium (Kenerley & Rogers 1976). Germinated ascospores were transferred to 2% oatmeal agar (OA, Difco), Leonian's medium (Booth 1971), and half strength potato dextrose agar (PDA, Difco). Asci and ascospores from dried specimens and conidia from pure cultures were examined in water or 5% KOH with Melzer's reagent.

For DNA sequencing, mycelium was harvested from the cultures, dried, then ground in 400 µL lysis buffer (Qiagen, USA) with a plastic pestle followed by incubation for 2 hr at 55°C Then 220 µL lysed solution was loaded into the QIAxtractor Robot (Qiagen, USA) and DNA extraction performed with a Qiagen DX reagent pack and tissue extraction protocol. DNA sequences for ITS, β-tubulin, and RPB2 were generated using the amplification primers from Hsieh et al. (2010). In addition, DNA was extracted from the hymenium of a fruit body from PDD 107494, prior to the specimen being dried for storage, and an ITS sequence generated.

The DNA sequences were concatenated and a phylogenetic analysis performed using our newly generated sequences together with selected taxa from U'Ren et al. (2016) and previously unpublished sequences from a culture grown from a collection of *Kretzschmariella culmorum* (Guadeloupe, on culm of bamboo, coll. J. Vivant, 1 Nov 1992, sent by H.W. Ravenel (#2505) to WSP; culture stored at The Institute of Plant and Microbial Biology, Academia Sinica). An initial analysis showed that *Entalbostroma erumpens* was within the *Xylaria* 'HY' clade of U'Ren et al. (2016). The taxa selected were those that represented the diversity across that clade, together with representative taxa from the other clades recognised within the Xylarioideae by U'Ren et al. *Poronia pileiformis* and *Podosordaria mexicana* were used as the outgroup. The sequences were aligned using MAFFT as implemented in Geneious R9 (Kearse et al. 2012). A Bayesian phylogenetic tree was generated using MrBayes 3.2 (Ronquist et al. 2012) with gaps treated as missing data, applying the GTR+I+G model for all three genes, the same model selected for all genes using the Akaike information criterion method in MrModeltest 2.3 (Nylander 2004). The data set was run with four chains for 10 million generations, trees sampled every 1000 generations with a burn-in of 25%. Bayesian posterior probabilities were obtained from 50% majority rule consensus trees.

Taxonomy

Entalbostroma J.D. Rogers & P.R. Johnst., **gen. nov.**

MycoBank MB817225

Differs from *Kretzschmariella* in having typical xylariaceous conidia.

Type: *Entalbostroma erumpens* J.D. Rogers & P.R. Johnst.

Etymology: For the white entostroma.

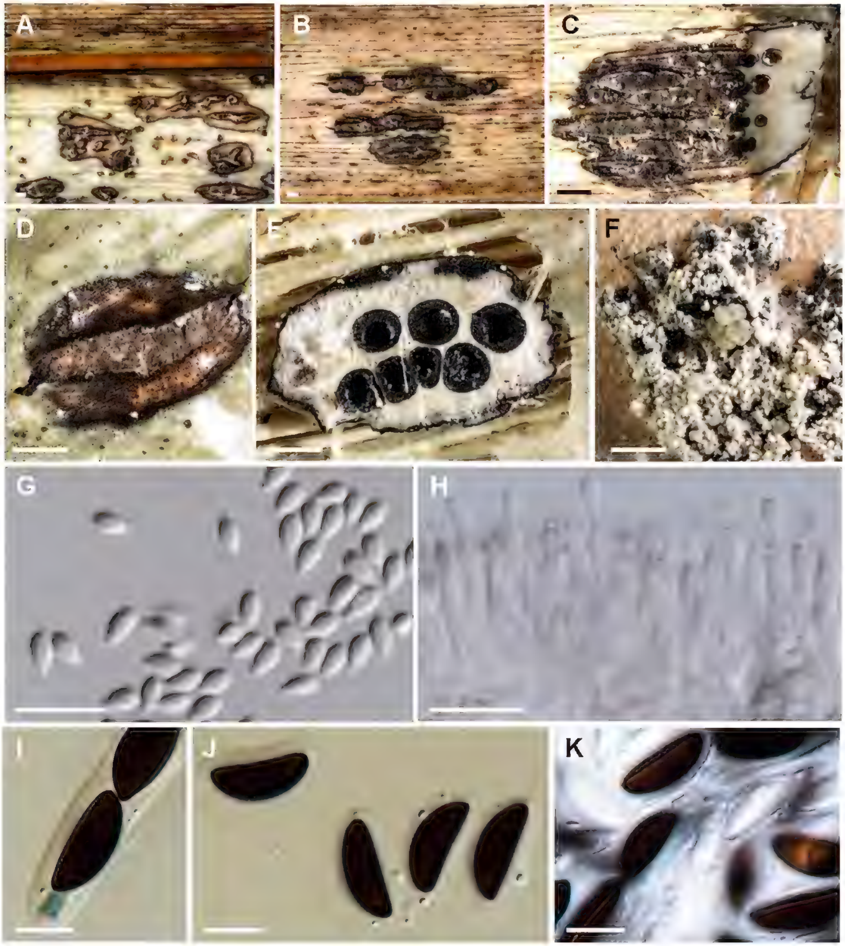


Figure 1 *Entalbostroma erumpens* (A–C, holotype, PDD 107494; D, E, I–K, PDD 107550; F–H, ICMP 21152). **A, B**, fruiting bodies erumpent from leaf, variable in shape. **C**, fruiting body in section showing white internal tissue and continuous narrow black outer layer. **D**, erumpent, partly exposed fruiting body showing barely differentiated ostioles. **E**, fruiting body from D with upper part removed to show internal structure; **F**, detail of surface of culture on PDA, darkened structures, and powdery layer of conidia in places. **G**, conidia from PDA culture. **H**, conidiogenous cells from PDA culture. **I**, ascus apex in KOH and Melzer's reagent. **J**, ascospores with gelatinous sheath and small apical gelatinous cap. **K**, ascospores with spore-length germ slit. Scale bars: A–F = 1 mm; G–K = 10 µm.

Stromata pulvinate to applanopulvinate, orbicular to elliptical to irregular, solitary to gregarious, erumpent becoming superficial; surface blackish, interior perithecium-bearing tissue white. Perithecia more or less spherical;

ostioles ill defined. Ascus apical ring inverted hat-shaped, bluing in Melzer's iodine reagent. Ascospores dark brown, with germ slit on flattened side, a hyaline gelatinous sheath. Paraphysate.

In culture, conidiophores forming a palisade-like layer of short, cylindric cells with conidia produced holoblastically on several scars near apex. Conidia hyaline, smooth, ellipsoid with narrowed flattened bases.

Entalbostroma erumpens J.D. Rogers & P.R. Johnst., **sp. nov.** FIG. 1

MYCOBANK MB817226

Differs from *Xylaria amphithele* by its initially immersed, then erumpent, non-stipitate ascomata, its non-erumpent perithecia, and its ascospores being surrounded by a gelatinous sheath.

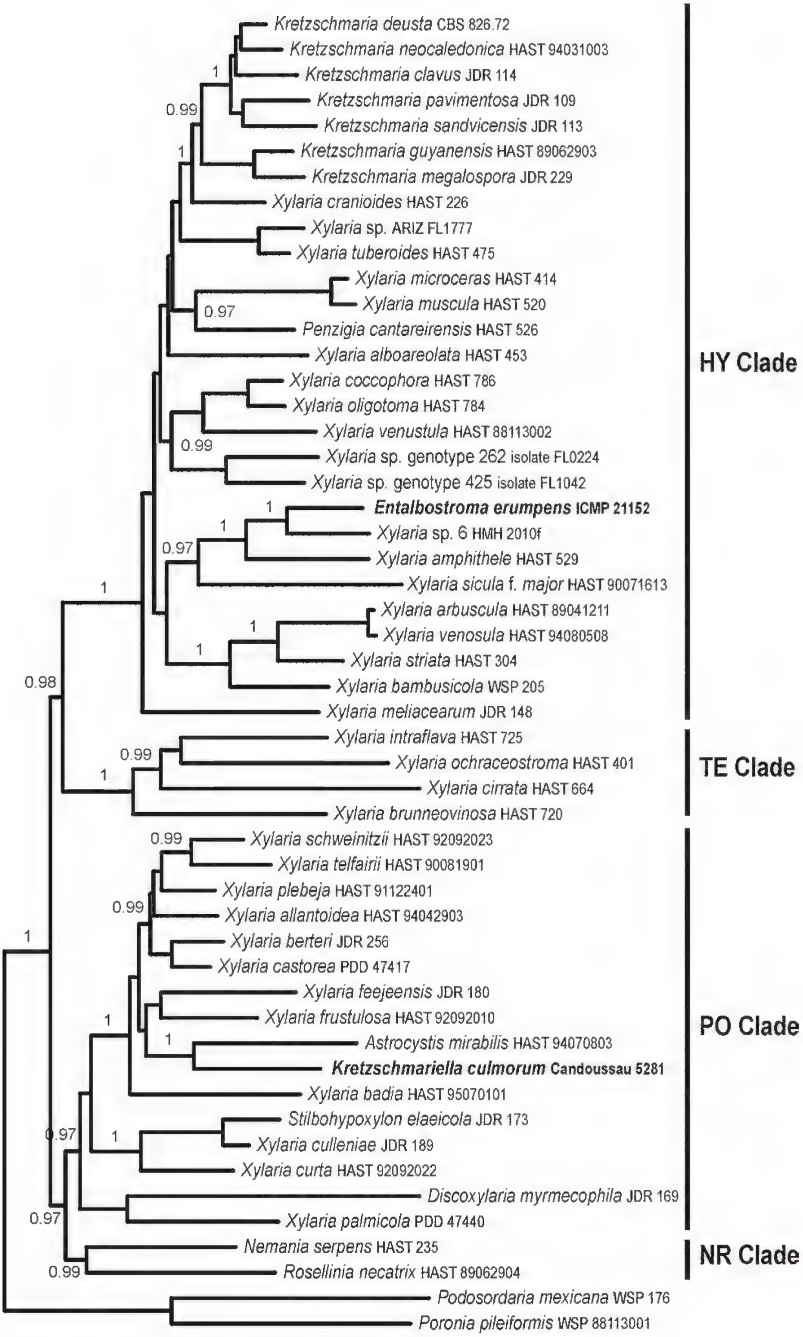
TYPE: New Zealand: Auckland: North Piha, Laird Thomson Track, on dead leaf *Phormium tenax* J.R. Forst. & G. Forst., 28 Aug 2015, coll. N.A. Martin (**Holotype**, PDD 107494; **isotype**, WSP 72775; ex type culture, ICMP 21152; GenBank KX258204, KX258205, KX258206).

ETYMOLOGY: For the erumpent nature of the ascomata from leaf tissue.

Stromata pulvinate to applanopulvinate, orbicular to elliptical to irregular, solitary to gregarious, erumpent becoming superficial, 2–10 mm long × 1–3 mm broad × 1–2 mm thick; surface blackish, interior perithecium-bearing tissue white. Perithecia more or less spherical, 0.2–0.4 mm diam. Ostioles ill defined. Asci short-stipitate, 120–146 × 8 µm total length. Ascus apical ring inverted hat-shaped, bluing in Melzer's iodine reagent, ca. 4.5 µm high, 2.7 µm broad. Ascospores dark brown, ellipsoid-inequilateral, 16–19 × 8–8.8 µm, with long germ slit on flattened side, with a hyaline gelatinous sheath that is often broader on the flattened side, and often with a small gelatinous cap at one end. Paraphysate.

Cultures from multiple ascospores on OA under 12 hr fluorescent light and 12 hr dark white to pinkish, covering 9 cm diam plate in 2–3 wk. Surface at first plane, appressed and smooth after 4–6 wk showing small areas of raised pimples. Pimples becoming enlarged and blackened, hollow. Conidiophores forming a palisade-like layer of short, cylindric cells with conidia produced holoblastically on several scars near apex. Conidia hyaline, smooth, ellipsoid with narrowed flattened bases indicating point of attachment to conidiogenous cell, (3–)4–5(–7) × 2–2.5 µm.

FIGURE 2. Bayesian phylogenetic tree based on concatenated ITS, β-tubulin, and RPB2 sequences, Bayesian posterior probabilities >0.95 on edges. Taxa selected from U'Ren et al. (2016) are labelled with voucher numbers; *Podosordaria mexicana* and *Poronia pileiformis* were selected as outgroup. Clade designations follow U'Ren et al. (2016). *Entalbostroma erumpens* sequences are deposited in GenBank as KX258204, KX258205, and KX258206; those from *Kretzschmariella culmorum* as KX430043, KX430045, and KX430046.


Kretzschmaria deusta CBS 826.72
Kretzschmaria neocaledonica HAST 94031003
Kretzschmaria clavus JDR 114
Kretzschmaria pavimentosa JDR 109
Kretzschmaria sandvicensis JDR 113
Kretzschmaria guyanensis HAST 89062903
Kretzschmaria megalospora JDR 229
Xylaria cranioides HAST 226
Xylaria sp. ARIZ FL1777
Xylaria tuberoides HAST 475
Xylaria microceras HAST 414
Xylaria muscula HAST 520
Penzigia cantareirensis HAST 526
Xylaria alboareolata HAST 453
Xylaria coccophora HAST 786
Xylaria oligotoma HAST 784
Xylaria venustula HAST 88113002
Xylaria sp. genotype 262 isolate FL0224
Xylaria sp. genotype 425 isolate FL1042
Entalbostroma erumpens ICMP 21152
Xylaria sp. 6 HMH 2010f
Xylaria amphithele HAST 529
Xylaria sicula f. major HAST 90071613
Xylaria arbuscula HAST 89041211
Xylaria venosula HAST 94080508
Xylaria striata HAST 304
Xylaria bambusicola WSP 205
Xylaria meliacearum JDR 148
Xylaria intraflava HAST 725
Xylaria ochraceostroma HAST 401
Xylaria cirrata HAST 664
Xylaria brunneovinosa HAST 720
Xylaria schweinitzii HAST 92092023
Xylaria telfairii HAST 90081901
Xylaria plebeja HAST 91122401
Xylaria allantoidea HAST 94042903
Xylaria berteri JDR 256
Xylaria castorea PDD 47417
Xylaria feejeensis JDR 180
Xylaria frustulosa HAST 92092010
Astrocystis mirabilis HAST 94070803
Kretzschmariella culmorum Candoussau 5281
Xylaria badia HAST 95070101
Stilbohypoxylon elaeicola JDR 173
Xylaria culleniae JDR 189
Xylaria curta HAST 92092022
Discoxylaria myrmecophila JDR 169
Xylaria palmicola PDD 47440
Nemania serpens HAST 235
Rosellinia necatrix HAST 89062904
Podosordaria mexicana WSP 176
Poronia pileiformis WSP 88113001
HY Clade
TE Clade
PO Clade
NR Clade
1
0.99
1
0.97
0.99
1
1
0.97
1
1
1
0.98
0.99
1
0.99
0.99
1
1
1
1
0.97
0.97
0.99

Additional Specimens examined: NEW ZEALAND: Northland: Poor Knights Islands, on dead leaves of *Phormium tenax*, 3 Sep 1984, coll. R.E. Beever (PDD 46231). Auckland: Piha South, Lookout Track, on dead leaves of *Phormium tenax*, 28 Aug 2015, coll. N.A. Martin (PDD 107553);. Muriwai, Quarry Track, on dead leaves of *Phormium tenax*, 21 July 2015, coll. N.A. Martin (PDD 107552); Muriwai, Maori Bay Track, on dead leaves of *Phormium tenax*, 21 Aug 2015, coll. N.A. Martin (PDD 107550); Waitakere Ranges, Nihotupu Dam, on dead leaves of *Phormium tenax*, 4 Nov 1960, coll. J.M. Dingley (PDD 42824). Mid Canterbury: Bealey Valley, on dead leaves of *Phormium cookianum* Le Jol. (as *P. colensoi* Hook. f.), 11 Mar 1983, coll. E. Horak (PDD 93072). Stewart Island: Doughboy Bay, on dead leaves of *Phormium* sp., 29 Apr 2002, coll. P.R. Johnston, S. Whitton, R. Leschen (PDD 107551).

Phylogeny

The ITS sequences from the fruit body from the PDD 107494 fungarium specimen and from cultures derived from that specimen (ICMP 21152) were identical. The DNA sequences from ICMP 21152 have been deposited in GenBank as KX258204, KX258205, and KX258206.

Entalbostroma erumpens formed a strongly supported sister relationship with *Xylaria amphithele* and *Xylaria* sp. 6 sensu Hsieh et al. (2010) within the 'HY' clade of U'Ren et al. (2016) (Fig 2). Morphologically it is divergent from *X. amphithele* (San Martín González & Rogers 1989), but it shares a leaf-inhabiting ecology with both this species and *Xylaria* sp. 6 (Hsieh et al. 2010). *Kretzschmariella* is phylogenetically distant, positioned within the 'PO' clade of U'Ren et al. (2016).

Discussion

The morphology of the sexual state of *Entalbostroma* recalls *Kretzschmariella*, both genera having erumpent ascomata with a thin black crust and white soft entostromatic tissue surrounding the perithecia. The two genera differ in their anamorph, our new fungus having a reduced *Geniculosporium*-like anamorph in culture (typical of species of *Xylaria* and *Kretzschmaria*), rather than the *Mirandina*-like conidial state of *Kretzschmariella* (Ju & Rogers 1994). This reinforces earlier studies showing that the morphology of the anamorph may be phylogenetically informative among these fungi. Inclusion of morphologically divergent taxa such as *Entalbostroma* will be necessary for future phylogenetic resolution of generic level clades within the *Xylariaceae*, and for recognition of the characters that can be used to recognise those clades morphologically.

Entalbostroma erumpens is geographically widespread on dead, partly decomposed leaves of *Phormium* spp. in New Zealand, and appears to be quite common on its host.

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